Structure of Two New Sesquiterpenoid Insect Antifeedants from *Celastrus rosthornianus*

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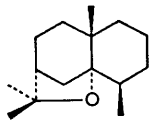
Two new sesquiterpene polyol esters have been isolated from the root bark of *Celastrus rosthornianus*. Their structures were elucidated, on the basis of ^{1}H NMR, ^{13}C NMR, NOESY, ^{1}H–^{13}C chemical-shift correlation, ^{1}H–^{13}C long range correlation (COLOC) and mass spectrometry, as 1β-acetoxy-2β,8β,9α-tribenzoyloxy-4α,6α-dihydroxy-β-dihydroagarofuran **3** and 1β-acetoxy-8β,9α-dibenzoyloxy-2β-(furan-β-carbonyloxy)-4α,6α-dihydroxy-β-dihydroagarofuran **4**. The complete assignment of ^{13}C NMR chemical shifts of compounds **3** and **4** on the basis of ^{1}H–^{13}C chemical-shift correlation spectral data and insecticidal tests on compound **3** against the larvae of *Pieris rapae* were also carried out.

Plants of the Celastraceae family are distributed in all parts of China; some of these plants were traditionally used in China to protect other plants from insect damage.[1] Various β-dihydroagarofuran, **1**, sesquiterpene polyol esters, including alkaloids, non-alkaloids and lactones, have been isolated from this family of plants.[2–4] Some of these sesquiterpene polyol esters exhibit insect antifeedant and/or insecticidal effects.[5–7] Generally, the bioactive constituents isolated from these insecticidal plants are alkaloids.[5,6] However, Wakabayashi *et al.* reported in 1988 that the non-alkaloid compound also showed insect antifeedant properties.[1] In our recent investigation on chemical constituents from the Celastraceae, two new sesquiterpene, non-alkaloidal, polyol esters, compounds **3** and **4**, were isolated from the root



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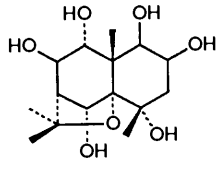

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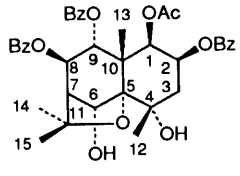

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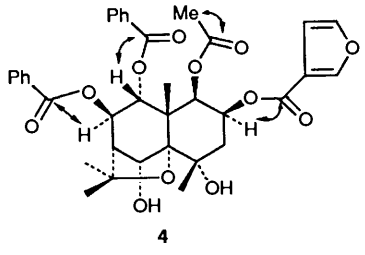

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bark of *Celatrus rosthornianus Loes.* Both compounds are derivatives of the polyol **2**. A preliminary bioactive test on compound **3** showed insect antifeedant effect against the larvae of *Pieris rapae*. In this paper, the structure elucidation and ^{13}C NMR chemical-shift assignment of compounds **3** and **4**, and the bioactivity test (see Experimental section) of compound **3** are presented.

Compound **3** analysed for $C_{38}H_{40}O_{11}$ by elementary analyses and mass spectrometry. Its IR spectrum revealed absorptions for a phenyl group at ν 1455 and 1602 cm^{-1}, an ester group at ν 1735 cm^{-1}, and free hydroxy groups at ν 3423 and 3486 cm^{-1}. The MS exhibited peaks due to the loss of acetic acid [*m/z* 612 (M − AcOH)] and benzoic acid [*m/z* 550 (M − $PhCO_2H$)]. In good agreement with the data above, the ^{1}H and ^{13}C NMR spectra suggested the presence of one acetate ester [δ_H 1.57 (3 H, s); δ_C 20.3 (Me) and 169.5 (CO_2)] and three benzoate esters [δ_H 7.48–8.08 (15 H, m); δ_C 128.3–133.6 (m, Ph) and 2 × 164.5 and 164.6 (3 × CO_2)]. In addition, the ^{13}C NMR and DEPT spectra indicated that the parent skeleton consisted of fifteen carbons: four methyl carbons (δ_C 21.7, 25.2, 26.2 and 30.5), one methylene carbon (δ_C 40.9), six methine carbons (δ_C 54.5, 69.0, 70.1, 75.0, 76.0 and 76.4) and four quaternary carbons (δ_C 49.5, 72.2, 84.2 and 91.8). These data were in good agreement with the spectral values assigned to the 1,2,4,6,8,9-hexasubstituted β-dihydroagarofuran parent skeleton.[7–11] Moreover, the molecular composition suggested the presence of two free hydroxy groups, which was confirmed by the fact that interchange with D_2O resulted in the disappearance of two signals, at δ_H 3.35 (1 H, s) and 5.00 (1 H, d, *J* 5.4 Hz). One free hydroxy group was located at C-4 because in all compounds of this class the 4-OH is free;[2,7] the second free OH group and the four esters mentioned above were located at C-1, C-2, C-6, C-8 and C-9 of β-dihydroagarofuran.

In the ^{1}H NMR spectrum (see Experimental section), the doublet at δ_H 5.62 (1 H, *J* 3.5 Hz) and the quartet at δ_H 5.89 (1 H, *J* 3.5, 6.8 Hz) were assigned to 1-H and 2-H, respectively. Generally, 1-H and 6-H in this class of compounds have axial stereochemistry,[2,7] thus the coupling constant ($J_{1,2}$ 3.5 Hz) suggested that 2-H had equatorial stereochemistry. In the NOESY spectrum, correlative signals between 4-OH (δ_H 3.35, 1 H, s) and 12-H_3 (δ_H 1.93, 3 H, s), between 6-OH (δ_H 5.00, 1 H, d, *J* 5.4 Hz) and 15-H_3 (δ_H 1.68, 1 H, s), between 8-H (δ_H 5.60, 1 H, d, *J* 3 Hz) and 14-H_3 (δ_H 1.71, s), between 6-H (δ_H 5.12, 1 H, d, *J* 5.4 Hz) and 12-H_3, between 6-H and 13-H_3 (δ_H 1.88, 1 H, s), and between 9-H (δ_H 5.19, 1 H, s) and 13-H_3 were found. This suggested the stereochemistry of equatorial hydrogens 8-H and 9-H, and confirmed the assignments mentioned above from ^{1}H NMR chemical shifts for 4- and 6-OH, 6, 8- and 9-H and 12-, 13-, 14- and 15-H_3. In particular, the weak coupling between 7-H^e and 6-H^a could be found in nearly all compounds of this class,[2]

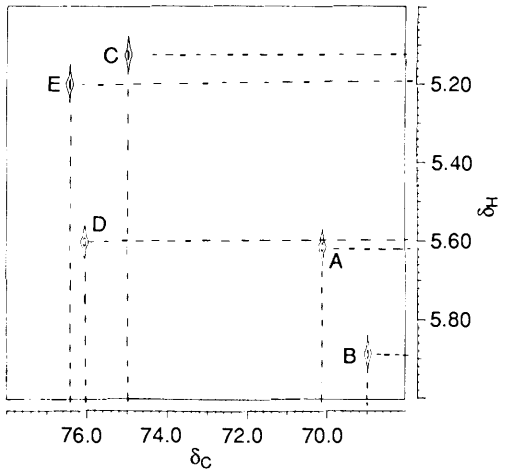

Fig. 1 Partial ^1H–^{13}C chemical-shift correlation spectrum of compound **3** (500 MHz: $CDCl_3$). Points A, B, C, D and E indicate the correlation between 1-H and C-1, 2-H and C-2, 6-H and C-6, 8-H and C-8 and 9-H and C-9, respectively.

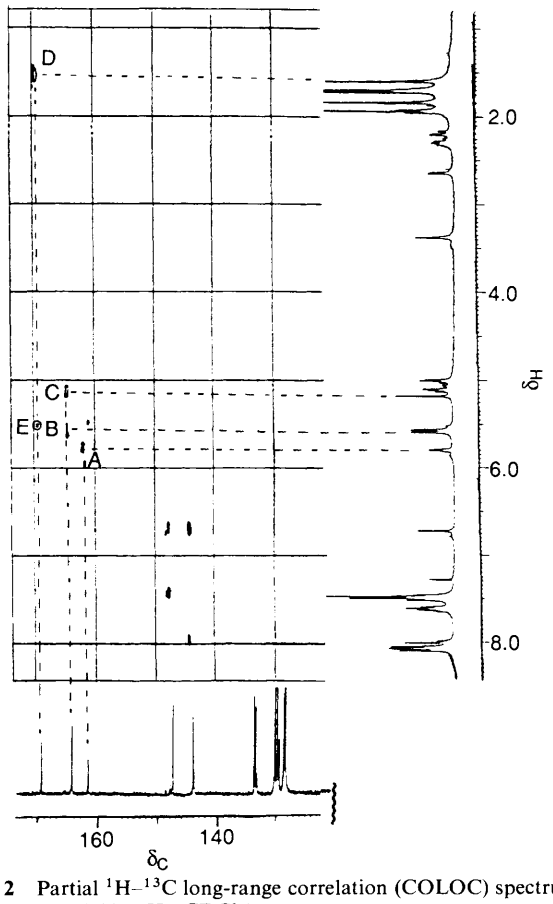

Fig. 2 Partial ^1H–^{13}C long-range correlation (COLOC) spectrum of compound **4** (400 MHz; $CDCl_3$)

and weak coupling between 8-H^e and 9-H^e had also been reported.[12–14] As described above for the NOESY spectrum, the second free hydroxy group was determined as being located at C-6, which was confirmed by the fact that, when subjected to interchange with D_2O, compound **3** gave only one ^1H NMR singlet for 6-H, at δ_H 5.12, instead of the AB quartet at δ_H 5.12 and 5.00 (J 5.4 Hz) for 6-H and 6-OH in the original spectrum. In addition, such a large coupling constant (J 5.4 Hz) between 6-H and 6-OH was also found in our previous investigation.[14]

The remaining problem was the location of four ester groups. In the ^1H NMR spectrum, the upfield and very sharp singlet at δ_H 1.57 (3 H) was assigned to acetate methyl; such a small chemical shift suggested that this acetate ester and one benzoate ester were located at C-1 and C-9 (or C-9 and C-1), respectively.[7] Furthermore, the mass spectrum exhibited an intensive fragment m/z 202, which analysed for $C_{13}H_{14}O_2$ and suggested that the benzoate ester was located at C-9.[7] Thus the acetate ester was located at C-1. Based on the above evidence, the structure of compound **3** was elucidated as 1β-acetoxy-2β,-8β,9α-tribenzoyloxy-4α, 6α-dihydroxy-β-dihydroagarofuran.

In general, the complete assignment of ^{13}C NMR chemical shifts for the parent skeleton on the basis of the ^{13}C NMR spectrum itself was very difficult, due to the fact that the methine carbons bearing ester groups or the methyl carbons, such as C-12 and C-14, in compound **3**, had very similar chemical shifts. However, the complete assignment (see Experimental section) of the ^{13}C NMR chemical shifts for compound **3** on the basis of ^1H–^{13}C chemical-shift correlation spectrum (Fig. 1) was easily carried out.

Compound **4** analysed for $C_{36}H_{38}O_{12}$ by elementary analyses and mass spectrometry. Its IR, MS, and ^1H and ^{13}C NMR spectral data (see Experimental section) suggested that compound **4** contained one acetate ester, two benzoate esters, one furan-β-carboxylate ester, and the 1,2,4,6,8,9-hexasubstituted β-dihydroagarofuran skeleton. In addition, the molecular composition suggested the presence of two free hydroxy groups. As with compound **3**, one free hydroxy group was located at C-4; the second was located at C-6 on the basis that interchange with D_2O caused the ^1H NMR AB quartet at δ_H 5.09 and 4.99 for 6-H and 6-OH to turn into one singlet at δ_H 5.09. Therefore, four ester groups were located at C-1, C-2, C-8 and C-9. In comparison with compound **3**, both compounds had very similar ^1H NMR spectral data in terms of coupling patterns and coupling constants, suggesting that compound **4** had the same stereochemistry at C-2, C-8 and C-9 as had compound **3**. The assignment of ^1H NMR and ^{13}C NMR chemical shifts for compound **4** was carried out on the basis of comparison with data for compound **3**.

The location of the four esters mentioned above was mainly based on a ^1H–^{13}C long-range correlation (COLOC) spectrum (Fig. 2), in which the correlative signals between 2-H (δ_H 5.79) and the carbonyl (δ_C 161.8) of furan-β-carboxylate ester (A), between 8-H (δ_H 5.58) and the carbonyl (δ_C 164.5) of one benzoate ester (B) and between 9-H (δ_H 5.17) and the carbonyl (δ_C 164.5) of the second benzoate ester (C), were found. This fact suggested the location of a β-furan-β-carbonyloxy group at C-2 and two benzoyloxy groups at C-8 and C-9, respectively. Therefore, the remaining acetate ester was located at C-1, which was confirmed by the upfield and sharp ^1H NMR singlet (δ_H 1.57, 3 H) of the acetate methyl and the intensive MS fragment m/z 202. In the COLOC spectrum, the correlative signal (E) between 1-H (δ_H 5.55) and the carbonyl (δ_C 169.5) of the acetate ester was not obvious. However, the correlative signal (D) between the sharp singlet (δ_H 1.57, 3 H) and the carbonyl (δ_C 169.5) of the acetate ester could be found, which confirmed our reasonable assignment of the sharp singlet at δ_H 1.57 to the acetate methyl. As a result, the structure of compound **4** was elucidated as 1β-acetoxy-8β,9α-dibenzoyloxy-2β-(furan-β-carbonyloxy)-4α,6α-dihydroxy-β-dihydroagarofuran.

Experimental

The m.p. of compound **3** was obtained on a Kofler apparatus (uncorr.). IR spectral data were obtained on an FT-5DX instrument with KBr discs. MS data were obtained on a VG ZAB-HS instrument operating at 70 eV. ^1H NMR, ^{13}C NMR, NOESY and COLOC spectra were obtained on a Bruker AM-400 instrument (^1H–^{13}C chemical-shift correlation spectrum was obtained on a Bruker AM-500 instrument) with $CDCl_3$ as solvent and $SiMe_4$ as internal standard. Elementary analyses

were carried out on a MOD1106 instrument. Silica gel (200–300 mesh) and neutral Al_2O_3 (100 mesh) were used for column chromatography. Merck silica gel 60 F254 and Merck RP-18 preparative plates were used for preparative chromatography. Detection of components was with a UV lamp. Experimental material was collected from Yunnan Province (China) and authenticated at the Department of Botanical Systematic, Kunming Institute of Botany, the Academy of Science of China. Voucher specimens are deposited at the Botanical Garden of Kunming Institute of Botany. Me_2CO was used for the control group in the bioactivity test.

Extraction and Isolation.—The air-dried root bark (2 kg) of *C. rosthornianus* was pulverized and extracted with acetone at room temperature for two days. Removal of the solvent under reduced pressure gave a dark brown residue, which was chromatographed on a neutral Al_2O_3 column with $CHCl_3$ as eluent to give a total fraction. After concentration of the total fraction under reduced pressure, the residue was rechromatographed on a silica gel column with Me_2CO–light petroleum (1:9→1:1) as eluent to give 48 fractions, which were combined into three groups. The middle polar group was chromatographed on preparative silica gel plates with Me_2CO–benzene (1:4) as developer, and finally purified on RP-18 plates with MeOH–water (4:1) as developer to yield compounds **3** (350 mg) and **4** (108 mg).

Compound **3**. Crystals, m.p. 245–246 °C (from MeOH); ν_{max}/cm^{-1} 3486 and 3423 (OH), 3064, 2945 and 2868 (Me), 1735 (C=O), 1602 and 1455 (Ph), 1370 and 1285 (CMe_2) and 1110, 1025, 962, 850 and 709; *m/z* (%) 672 (2, M^+), 657 (4, M − Me), 639 (3, 657 − H_2O), 612 (2, M − AcOH), 550 (3, M − $PhCO_2H$), 535 (23, 657 − $PhCO_2H$), 475 (2, 535 − AcOH), 428 (3, 550 − $PhCO_2H$), 413 (2, 657 − 2 × $PhCO_2H$), 202 (25), 105 (100, PhCO), 77 (25, Ph) and 43 (24, Ac) (Found: C, 67.85; H, 5.9. $C_{38}H_{40}O_{11}$ requires C, 67.86; H, 5.95%); δ_H 5.62 (1 H, d, *J* 3.5 Hz, 1-H), 5.89 (1 H, dd, *J* 3.5, 6.8 Hz, 2-H), 2.20–2.31 (2 H, m, 3-H_2), 5.12 (1 H, d, *J* 5.4 Hz, 6-H), 5.00 (1 H, d, *J* 5.4 Hz, 6-OH), 2.63 (1 H, d, *J* 3 Hz, 7-H), 5.59 (1 H, d, *J* 3 Hz, 8-H), 5.19 (1 H, s, 9-H), 3.35 (1 H, s, 4-OH), 1.93 (3 H, s, 12-H_3), 1.88 (3 H, s, 13-H_3), 1.71 (3 H, s, 14-H_3), 1.68 (3 H, s, 15-H_3), 1.57 (3 H, s, OAc) and 7.48–8.03 (15 H, m, Ph); δ_C 70.1 (C-1), 69.0 (C-2), 40.9 (C-3), 72.2 (C-4), 91.8 (C-5), 75.0 (C-6), 54.5 (C-7), 76.0 (C-8), 76.4 (C-9), 49.5 (C-10), 84.2 (C-11), 25.2 (C-12), 21.7 (C-13), 26.2 (C-14), 30.5 (C-15), 20.3 and 169.5 (AcO) and 128.3–133.6, 2 × 164.5 and 165.7 (3 × PhCO).

Compound **4**. Amorphous, white powder, ν_{max}/cm^{-1} 3521 and 3409 (OH), 2938 (Me), 1729 (C=O), 1581 and 1454 (aromatic), 1363 and 1272 (CMe_2) and 1096, 1025, 970 and 709; *m/z* (%) 662 (2, M^+), 647 (4, M − Me), 629 (3, 647 − H_2O), 602 (2, M − AcOH), 550 (2, M − furan-β-carbonic acid), 535 (3, 647 − furan-β-carbonic acid), 525 (21, 647 − $PhCO_2H$), 507 (2, 629 − $PhCO_2H$), 465 (2, 525 − AcOH), 428 (3, 550 − $PhCO_2H$), 202 (25), 105 (100, PhCO), 95 (30, furan-β-carbonyl), 77 (47, Ph) and 43 (10, Ac) (Found: C, 65.2; H, 5.7. $C_{36}H_{38}O_{12}$ requires C, 65.26, H, 5.74%); δ_H 5.55 (1 H, d, *J* 3.8 Hz, 1-H), 5.79 (1 H, dd, *J* 3.8, 6.8 Hz, 2-H), 2.14–2.27 (2 H, m, 3-H_2), 5.09 (1 H, d, *J* 5.3 Hz, 6-H), 4.99 (1 H, d, *J* 5.4 Hz, 6-OH), 2.63 (1 H, d, *J* 3.1 Hz, 7-H), 5.58 (1 H, d, *J* 3.1 Hz, 8-H), 5.17 (1 H, s, 9-H), 3.33 (1 H, s, 4-OH), 1.92 (3 H, s, 12-H_3), 1.82 (3 H H, s, 13-H_3), 1.70 (3 H, s, 14-H_3), 1.67 (3 H, s, 15-H_3), 1.57 (3 H, s, OAc), 7.45–8.08 (10 H, m, PhCO), and 6.71, 7.48 and 8.01 [3 × 1 H, 3 × br s, (furan-β-carboxylate hydrogens)]; δ_C 70.0 (C-1), 68.5 (C-2), 40.8 (C-3), 72.2 (C-4), 91.9 (C-5), 75.0 (C-6), 54.2 (C-7), 76.1 (C-8), 76.4 (C-9), 49.5 (C-10), 84.2 (C-11), 25.2 (C-12), 21.5 (C-13), 26.2 (C-14), 30.5 (C-15), 20.2 and 169.5 (AcO), 128.4–133.7, 2 × 164.5 (2 × PhCO) and 109.6, 119.1, 144.1, 147.5 and 161.8 (furan-β-carboxylate).

Bioactive Test for Compound **3**.—Test leaves of wild cabbage were macerated with an Me_2CO solution of the test sample (500 ppm) for a short while. After the leaves had been dried in air, larvae of *C. rapae* (previously weighed and denied food for 3 h) were placed on the leaves. The area of leaf eaten, the new weight of the larvae and the number of dead larvae were recorded. The antifeedant rate was calculated to be 49%.

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